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The Pathogenesis of Acute Diarrhoeal Disease in Early Life*

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Consecutive deaths of children in Guatemala City were investigated with a view to discovering possible correlations between malnutrition, enteric infection and diarrhoeal disease. Malnutrition and diarrhoeal disease were common in this childhood population. It was found that enteric pathogens did not occur in large enough numbers to constitute a major cause of diarrhoeal disease. Children with malnutrition and diarrhoeal disease not associated with enteric pathogens were noted to have a non-ulcerative inflammatory reaction in the jejunal mucosa. The jejunum of these children contained an abnormally large population of bacteria, not recognized as bacterial pathogens, and whose presence may be the basis of diarrhoeal disease in children suffering from malnutrition.

The author suggests that diarrhoeal disease in association with malnutrition be treated with antibiotics, fluids and electrolytes as a short-term measure, and by raising the nutritional standard as a long-term measure.

It has been the experience of many workers in the epidemiology of diarrhoeal disease that, in the younger age-groups, the percentage of patients with diarrhoeal disease who harbour specific bacterial enteric pathogens is low. Sabin (1963) has recently summarized reports supporting this observation. In the study to be described, less than half of the patients with diarrhoeal disease had enteric pathogens of any type, alone or in combination. Because the experience of others suggested a relatively small percentage of isolations for recognized enteric pathogens, a particular protocol designed to provide maximum opportunity for the isolation of bacterial, viral and other enteric pathogens was followed rigidly in our investigation of diarrhoeal disease in infants and children admitted to the Paediatric Service of the Roosevelt Hospital in Guatemala City. The study was carried out in the Departments of Paediatrics and Pathology of the Roosevelt Hospital, and in the laboratories of the Institute of Nutrition of Central America and Panama (INCAP). Invaluable support was provided by Dr Nevin Scrimshaw and Dr Carlos Tejada. Bacteriological and parasitological examinations were performed by Miss Virginia Pierce of the INCAP staff. Viral isolations and identifications and quantitative bacteriological studies were conducted at the Miami Regional Laboratory of the Florida State Board of Health under the direction of Dr Warren Hoffert. The collection of specimens from patients with diarrhoeal disease was directed by Dr Donald Feldman of the Pathology Department of the Peter Bent Brigham Hospital, and the Harvard Medical School. He performed all the post-mortem examinations and collected specimens for the isolation of all possible pathogens. For later reference, portions of the small and large intestine and their contents were frozen rapidly and maintained in the frozen state.

MATERIAL STUDIED

Guatemala was chosen for this detailed study of the factors responsible for acute diarrhoeal disease in early life because of the excellence of its clinical and laboratory facilities, and because diarrhoeal disease represents the major cause of mortality in this population. Since diarrhoeal disease is also a major cause of mortality in many of the developing countries, the implications of the study to be described may be pertinent to problems facing these countries.

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It was our plan to study consecutive deaths occurring in the Paediatric Service at the Roosevelt Hospital. This study of consecutive deaths was possible, and selection could be avoided, because permission for such study is implicit in the admission of patients to this hospital. Autopsies could be performed promptly and this was done, not only in accordance with the aims of this study but also because a delay in the examination might have interrupted the continuity of this series. In all, 63 deaths were investigated—about one half in the spring and the other half in the fall.

RESULTS

Only a summary of our findings and their implications can be presented in this report. Details are contained in reports to the Commission on Enteric Infections of the Armed Forces Epidemiological Board and in other reports to be published.

Malnutrition and diarrhoeal disease were common in this childhood population (Table 1). Of the 63 cases, 40 had gross and/or microscopic evidence of malnutrition and, of these, 35 had diarrhoeal disease. Fifty of the 63 cases had diarrhoeal disease, and 35 of these showed malnutrition. In only eight cases was neither malnutrition nor diarrhoeal disease present.

In Table 2 are listed the recognized or presumed bacterial and viral pathogens isolated. In 48% of the cases with diarrhoeal disease, none of these agents was isolated. Few pathogenic protozoa were identified: there were three cases with *Giardia lamblia* and none with *Entamoeba histolytica*. Helminths were common in faecal specimens, but not in sufficient numbers to be considered the basis of diarrhoeal disease; nor could any of the observed helminthic lesions be regarded as having this implication.

TABLE 1

RELATIONSHIP BETWEEN DIARRHOEAL DISEASE AND MALNUTRITION IN 63 CONSECUTIVE DEATHS STUDIED IN GUATEMALA CITY

Diarrhoeal disease	Malnutrition		
	Present	Absent	Total
Present	35	15	50
Absent	5	8	13
Total	40	23	63

TABLE 2

BACTERIAL AND VIRAL ISOLATIONS IN 63 CONSECUTIVE DEATHS STUDIED IN GUATEMALA CITY

Agents isolated	Percentage with diarrhoea (50 cases)	Percentage without diarrhoea (13 cases)
Shigellae, salmonellae, *E. coli* alone or with one another or viruses	46	23
Viruses alone or with bacteria	18	15
Viruses alone	6	7.7
Shigellae alone	12	0
Salmonellae alone	2	0
E. coli alone	18	0
Shigellae + viruses	2	0
Salmonellae+viruses	4	0
E. coli+viruses	6	7.7
No agents isolated	48	69

A correlation of bacterial pathogens with diarrhoeal disease (Table 3) shows that fewer cases had associated pathogens (23/50) than did not (27/50). This led us to a detailed analysis of mucosal alterations in the second group and of the total bacterial populations in their small intestines. From our study of a group of five cases with malnutrition but without diarrhoeal disease (Table 1), we concluded that advanced malnutrition with jejunal and pancreatic acinar atrophy could obtain in the absence of diarrhoeal disease. In such cases, the jejunal mucosa showed no inflammatory response (Fig. 1). However, when diarrhoeal disease occurred with malnutrition, even in the absence of a

TABLE 3

RELATIONSHIP BETWEEN DIARRHOEAL DISEASE AND BACTERIAL PATHOGENS IN 63 DEATHS STUDIED IN GUATEMALA CITY

Diarrhoeal disease	Bacterial pathogens		
	Present	Absent	Total
Present	23	27	50
Absent	3	10	13
Total	26	37	63

specific pathogen, an inflammatory response was noted in the jejunal mucosa (Fig. 2). (In Fig. 3, the jejunal mucosa of a normal infant is shown for comparison.) At this point, the total bacterial population became of interest. The bacterial populations of the proximal small intestine and its contents could not be determined in all cases, some specimens having been used up during viral studies. Cases were divided into two groups chosen arbitrarily—namely, those with more than 900 viable bacteria per gram of tissue and contents, and those with less. It should be noted that there may be a 10-fold to 100-fold reduction in viable bacteria when intestinal specimens are frozen and thawed before cultivation. Since the proximal jejunum is often sterile, and the specimens used for determining bacterial counts had been frozen upon collection at autopsy, the high counts obtained in cases with malnutrition and diarrhoeal disease appeared to be all the more significant.

Bacterial populations were determined by making serial 10-fold dilutions of the specimen and inoculating media for aerobic and anaerobic cultivation. Total bacterial counts were made per gram of specimen, and organisms were classified on the basis of morphology and Gram-staining characteristics only. Of 47 specimens so studied, 41 (87%) had Gram-positive cocci, 21 (45%) had Gram-positive bacilli, 6 (13%) had Gram-negative bacilli and 3 (6%) had Gram-negative cocci. Nine of these 47 specimens were from cases without diarrhoeal disease, and only one (11%) had a count of over 900 bacteria per gram. Of the 38 cases with diarrhoeal disease, 25 (66%) had counts of over 900 bacteria per gram, and most had counts of 4000 bacteria per gram and higher. Of the cases with high bacterial counts, less than one-third had associated bacterial enteropathogens. Although the number of cases studied was small, weight is added to the observation by the fact that seven specimens had only 0-100 viable bacteria per gram of specimen.

DISCUSSION

It should be noted that in a variety of gastrointestinal diseases there may be high bacterial populations in the jejunal lumen. Among these are chronic ulcerative colitis and cirrhosis of the liver, both of which may have associated recurrent diarrhoea. To what extent a high bacterial population in the jejunum may reflect disease in the gastrointestinal tract, or possibly be the basis for diarrhoeal disease, will have to be determined by a long-term study of a selected population of patients. Our observations in Guatemalan children, among whom malnutrition is common, suggest that malnutrition may be one of the factors favouring high bacterial populations in the jejunum. We have noted the association of malnutrition and diarrhoea with a high bacterial population and a non-ulcerative inflammatory reaction in the jejunum. It is the type of mucosal pattern that is observed in cholera (Gangarosa et al., 1960) and that is associated with massive loss of fluid and electrolytes. This coincidence suggests (*a*) that in childhood malnutrition there may be abnormally high bacterial populations in the jejunum, (*b*) that such populations may be the basis for mucosal inflammatory reactions of the type observed in cholera, and (*c*) that diarrhoea in malnutrition may result from abnormally large populations of bacteria not generally regarded as enteric pathogens. The latter mechanism is postulated by Bruch et al. (1963) as the basis for some cases of diarrhoeal disease in Guatemalan highland villages. Further studies are needed to determine whether the abnormally high populations of bacteria not regarded as enteric pathogens act directly on the intestine in inducing diarrhoea or whether an indirect mechanism involving anaphylaxis is responsible, as suggested by Thomlinson & Buxton (1963). In either case, it is desirable to reduce the bacterial population in this—so often sterile—portion of the intestine, by the use of antibiotics in the short run, and by improving the patient's nutrition in the long run.

One might speculate further on the effect of a high bacterial population in a jejunum with an atrophic mucosa and hence, presumably, with an ineffective absorbing surface. In these circumstances, a large number of bacteria together with the end-products of bacterial action on nutriments could increase the osmotic properties of the contents. This, combined with impaired absorption of the intestinal mucosa, could lead to the flow of abnormal amounts of fluid and electrolytes into the intestinal lumen, thus adding to the loss of fluid, electrolytes and nutriments through the intestinal tract.

RÉSUMÉ

Dans la ville de Guatemala, les maladies diarrhéiques représentent la principale cause de mortalité infantile. Pour tenter de préciser la pathogénie de ce type d'affection, l'auteur a mené des investigations d'ordre clinique, bactériologique et nécropsique, à la suite de 63 décès consécutifs survenus chez des enfants hospitalisés.

50 enfants avaient souffert de diarrhée, et chez 35 d'entre eux, elle était associée à un état de malnutrition avancé. Il ne semble pas que l'on puisse incriminer un agent infectieux spécifique et, dans 23 cas seulement, on a pu mettre en évidence la présence de germes pathogènes. Chez les enfants atteints de diarrhée endémique et souffrant de malnutrition, on constata des lésions inflammatoires non ulcératives de la muqueuse jéjunale avec, localement, une pullulation microbienne banale intense.

L'auteur pense que, chez l'enfant, la malnutrition favorise le développement de cette faune microbienne, dont la présence provoque la réaction inflammatoire de la muqueuse et l'apparition des symptômes de la maladie diarrhéique.

Il préconise comme mesures thérapeutiques immédiates l'emploi d'antibiotiques, la réhydratation pour compenser les pertes liquidiennes et, à long terme, l'amélioration de l'état de nutrition des patients.

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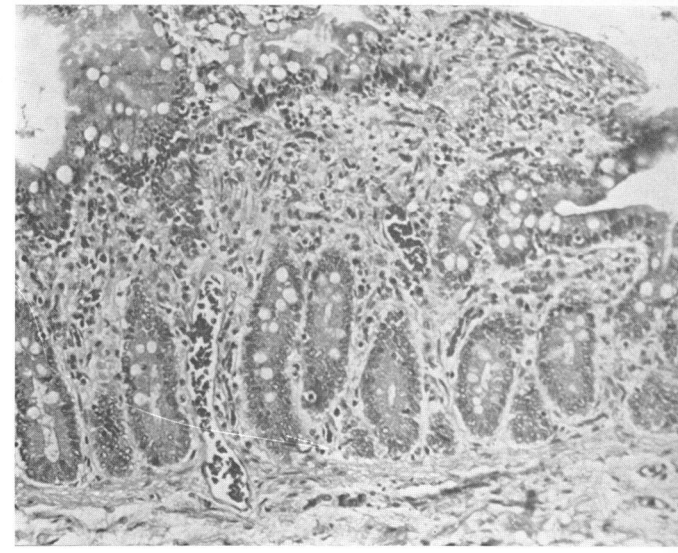
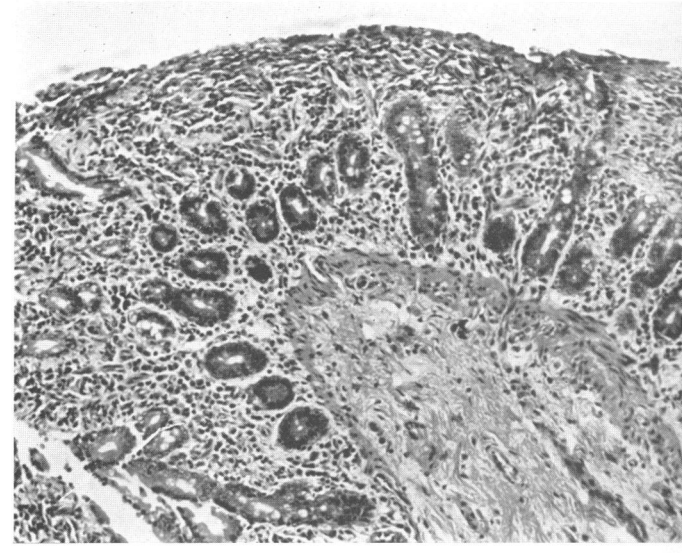
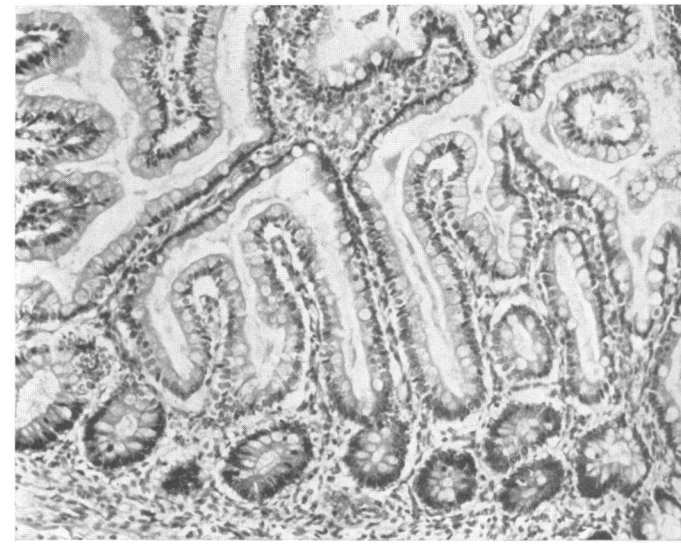
FIG. 1-3. JEJUNAL SPECIMENS [a]



1. Section of jejunum obtained from Guatemalan child with malnutrition but no diarrhoea. The structure of the villi is distorted and there is a reduced ratio of villus height to crypt depth. Note the sparse cellularity of the lamina propria and the complement of goblet cells.



2. Section of jejunum obtained from Guatemalan child with malnutrition and diarrhoea but without specific enteric pathogens. The increased cellularity of the lamina propria and the reduction of the number of goblet cells are evidence of inflammatory reaction in this atrophic mucosa.



3. Section of jejunum obtained from healthy child, showing normal ratio of villus height to crypt depth and normal complement of goblet cells and cells in the lamina propria.

[a] All three figures were photographed at the same magnification from histological preparations stained with haematoxylin and eosin. Tissue for these preparations was obtained from autopsy specimens.